Minutes of the VICH Biologicals Quality Monitoring Working Group, Mycoplasma

Topic Meeting Brussels, Belgium, November 15-16, 1999. The 14 issues for resolution collected by G. Christianson and distributed in June, 1999 were the focus of discussion.

1. Determine if the broth and agar culture method and one additional test method (DNA staining or PCR) will be required and whether some procedures will be screening and others confirmatory.

Agreed - The culture method is the fundamental method of mycoplasma detection. It is the method of general acceptance and experience. The use of multiple methods may improve detection sensitivity/specificity or find special application.

Action - WG will better define the 3 methods and then decide their use according to assay characteristics. A 2-day, EDQM-sponsored meeting on PCR testing of mycoplasma will be held in Paris during the period March 13-15, 2000, which may further clarify the method and applicability of the technique for consideration by the WG. Not all WG members will be able to participate in the meeting.

2. Determine if the guideline is a test for the "absence of Mycoplasma" or the "contamination by Mycoplasma".

Agreed - Phrase "Contamination by Mycoplasma" is acceptable in the guidelines.

3. Determine the method for validating laboratory method/operator/media for culturina, and the sensitivity of the PCR and DNA staining procedures.

See 1., above regarding PCR and DNA, and 3.a-h, j-1 below for culture.

a. Determine if the 5 strains (section 1.1.1) are sufficient to prove sensitivity or does a bovine *(M.* bovis or *M. ar-inintl* or additional species need to be added to the list of reference control organisms.

Agreed - Selected M. orale, A. laidlawii, M. synoviae, M. hyorhinus. If the material has no significant risk of exposure to avian origin ingredients, M. synoviae is exempted. If the material has no significant risk of exposure to mammalian origin ingredients, M. hyorhinus is exempted. If the material with risk only to avian origin ingredients has no antibiotics, A. laidlawii is exempted. The use of combinations of multiple media to satisfy the conditions is acceptable.

Action - EP to check if there is a reason for including M. gallisepticum and share info by Email. USDA to check if any mammalian mycoplasmas resemble M. synoviae growth characteristics and share info by Email.

b. Determine how the reference control organisms should be chosen to validate each of the test procedures (culture, DNA stain, or PCR).

See 3 and 3a.

c. Determine if color changing titer should be part of the media growth promotion requirements.

Agreed - Color indicators are not required. However, when a indicator is used, a subculture onto agar will be done whenever a color change in the indicator occurs, in addition to the routine subculturing schedule. If a color change occurs upon addition of product indicating a pH change from that addition, the pH should be corrected.

d. Determine if 15 subcultures should be allowed as the maximum passage number of the reference control organisms or should some other passage level be established.

Agreed - 15 in vitro passages from the field isolate will be specified as the maximum in the guideline.

e. Determine the passage number of those Mycoplasma strains specified in 1.1.1

Agreed - EDQM is beginning a project to produce low passage reference preparations of the VICH WG designated strains. New standards strains will be those exhibiting colonies upon initial subculture from the clinical specimen. If developed significantly before the implementation date of the guideline, the other region regulatory authorities will share the cost of development and use these preparations for implementing the guideline. The passage level of these future preparations will be sufficiently below the 15 passage maximum to allow distribution and expansion by testing laboratories. If laboratories wish to produce their own references of the strains they must provide the passage level.

f. Determine if the Mycoplasma species specified in section 1.1.1 are still available from the ATCC.

Agreed - Yes, they are still available as type culture reagents. They will only be used for identification purposes.

g. Determine if the ATCC cultures are guaranteed consistent between vials.

Agreed - No, but they will not need to be for the purposenow intended.

h. Determine the number of laboratories that need to be involved in certifying the reference control organisms.

Agreed - To be determined by characteristics found by originating laboratory.

i. Determine the number of media batches or PCR and DNA staining repetitions needed to certify them as consistently sensitive.

To be determined - after PCR and DNA staining procedures are better characterized.

j. Determine if and how reference control organisms could be used to maintain consistency after external laboratory validation.

Suggested protocol for further Email discussion:
Using a fixed technique, 5 levels of validation need to be addressed:

0 - Validation of technique/reference strains
    Ref Strain Master Preps titered by X labs in 3 regions
        X depends on characteristics shown by originating lab (EDQM)
1 -Validation (external) of the laboratory/operators
    Ref Strain Master Preps titered by each individual lab (few vials supplied by national lab)
        Result must be within variation derived from 3-region study
    Ref Strain Working Preps prepared from Ref Strain Master Prep
    Ref Strain Working Preps titered in same study as Ref Strain Master Preps, establishing their characteristics
2 -Validation of the media recipe/procedure -- after change in composition or media preparation method
    Fuse with level 1 (Master Preps only)
3 - Validation of media formulation -- after lot-change
    Fuse with level 4
4 -Validation of media batch
    Ref Strain Working Preps run on each media batch
        Result must be within variation derived from 3-region study and Working Prep study
5 -Validation of assay (test day)
    One Ref Strain Working Prep be run in each assay
        Result must be within variation derived from Working Prep study

Recipe/procedure - self explanatory
Formulation - the use of the recipe/procedure using unique lots of (critical) components
Batch - result of one production/mixing

k. Determine the maximum number of CFU (40 100 or ??) of the reference control organisms that will be used to test sensitivity of the test media or procedure.

Suggested compromise - 100 cfu.

4. Determine if section 1 1 4 (Inhibitory Substances) is to be required and if a test method will be specified.

See 8.

5. Determine if it is necessary to inoculate both liquid and solid media directly with the product to be examined.

Action - Members to gather data, share with WG, and agree by Email.

6. Determine if both aerobic (5% C02 in air) and microaerophilic (5% CO_2_ in 95% Nitrogen) will be required for afar plate incubation.

Suggested compromise - Only microaerophilic environment will be required for agar plate incubation. This will require further data distribution and discussion by Email.

7. Determine the volume of sample or subculture to be plated (0.1, 0.2, or 0.25 ml).

Suggested compromise -- 0.2 ml will be plated.

8. Determine if a 10 ml or 1 ml initial product inoculum, per test vessel, should be used.

Action -- The inoculum volume and the need for an inhibition test are linked. Each member will gather data and share with WG. Alternatives will be suggested and discussion will be continued on Email.

9. Determine the minimum number of days each agar plate should be incubated (10, 14, 21) before it is determined to be negative for mycoplasma growth.

Agreed - 14 days will be written into the guidelines. **Action** - EU will discuss and share with the WG info and data as to why the member states agree or disagree.

10. Determine if there should be retests and if so how many samples and what quantity of media and inoculum should be used for a retest.

Agreed - Upon finding a positive result on the original (1 g`) test, 1 retest (2"d test) with original technique may be performed. If a retest is not performed, the sample is considered contaminated. If retesting is done, and the retest (2°d test) is positive, the sample is considered contaminated with mycoplasma. If the retest (2" test) is negative, another retest (3`d test) must be performed. Laboratories may run two retests (2"d and 3rd tests) simultaneously to save time. If two or more of the three tests are positive, the sample is considered contaminated with mycoplasma.

11. Determine if the PCR and DNA staining methods should just be recommended or specified (how specific) in the guidelines

See 1.

12. Determine if cells types other than VEROs can be used in the DNA staining procedure.

Agreed - Other cells may be used if validated in the technique to have equal or greater sensitivity to Vero cells using the Reference Strains. The indicator cell line used should have a discrete nucleus with a minimum of extra nuclear chromatid DNA.

13. Determine from what substrate the PCR test is to be performed; directly from the product or from some broth or cell subculture.

Action - The members will gain experience in the technique and will share information with WG. A 2-day, EDQMsponsored meeting on PCR testing of mycoplasma will be held in Strasbourg during the period March 13-15, 2000.

14. Determine if a characterizing probe needs to be used to verify the 160 by amplicon in the PCR test.

Action -- The members will gain experience in the technique and will share information with WG. A less labor-intensive reporting system is desired.

Note: A discussion document will be drafted prior to the next WG meeting in the USA, **July 10-15, 2000. Note:** An introduction in line with other VICH guidelines will be written for this guideline.